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STUDIES ON PERENNIAL WEED CONTROL IN SOUTH DAKOTA

BY

STEVEN ROBERT GYLLING

A thesis submitted
in partial fulfillment of the requirements for the
degree Doctor of Philosophy
Major in Agronomy

South Dakota State University
1985

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STUDIES ON PERENNIAL WEED CONTROL IN SOUTH DAKOTA

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

W.E. Arnold Date
Thesis Adviser

Maurice L. Horton Date
Head, Plant Science Department

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SG

STUDIES ON PERENNIAL WEED CONTROL IN SOUTH DAKOTA

Abstract

STEVEN R. GYLLING

Studies were conducted to measure efficacy and economics of selected herbicide treatments for leafy spurge control in pasture, and to characterize the interaction of ammonium sulfate and glyphosate for quackgrass control. Several treatments resulted in leafy spurge control exceeding 90% in the tested pasture. Mean herbage dry-weight yield in treated plots was 2340 kg/ha, a 67% increase over untreated plots. Forage yields did not significantly differ among treatments controlling 90% or more leafy spurge. Marginal net return over marginal cost from herbicide treatments ranged from $35 to loss of $63/ha. Treatments providing satisfactory leafy spurge control with minimum economic risk were annual spring applications of 2,4-D at 1.7 kg/ha or dicamba + 2,4-D at 0.6 + 1.1 kg/ha, and biannual application of 2,4-D at 0.8 kg/ha. Addition of ammonium sulfate at rates of 1.4 to 5.6 kg/ha to glyphosate spray solutions of 0.22 to 0.68 kg/ha significantly improved quackgrass control over glyphosate alone at the same rates. An approximate doubling of activity was observed at low glyphosate rates. Generally any ammonium sulfate rate from 1.4 to 5.6 kg/ha produced similar effects. Since ammonium sulfate was not phytotoxic to quackgrass, the observed interaction between ammonium sulfate and glyphosate was an

enhancement of glyphosate by ammonium sulfate. Electrolyte leakage from quackgrass leaves treated with ammonium sulfate and glyphosate was greater than from leaves treated with glyphosate alone. Absorption of ^{14}C-glyphosate increased in solutions which contained ammonium sulfate. Translocation of ^{14}C-glyphosate was not significantly affected by ammonium sulfate. More plastoglobuli were found in chloroplasts of plants 80 hr after treatment with glyphosate plus ammonium sulfate than in those treated with glyphosate. Based on these tests, ammonium sulfate enhancement of glyphosate is most likely from increased absorption of glyphosate into quackgrass leaves caused by increased cell membrane permeability due to the ammonium sulfate.

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INTRODUCTION

Perennial weeds are often the most difficult and expensive weeds to control in farmland and pasture. Repeated cultivation can be successfully used to control perennial weeds, but is not a viable option in rangeland or reduced tillage cropping. While annual weed infestations may diminish under reduced tillage, perennial weeds are generally favored. One of the most serious pasture weeds in South Dakota is the perennial plant leafy spurge (Euphorbia esula L.). Quackgrass (Agropyron repens L.) is one of the most widely-distributed perennial weeds in South Dakota cropland.

Leafy spurge was first noted in South Dakota in 1902 (5), and has spread to occupy approximately 37,000 ha[1] in the state. Several herbicides can be used to control leafy spurge, but treatment costs vary considerably. While weed control in cropland is generally economically beneficial, many producers question whether perennial weeds such as leafy spurge can be economically controlled in pastures.

Quackgrass infests over 78,000 ha in South Dakota (13). Labeled herbicides which can effectively control quackgrass are glyphosate[2] [N-(phosphonomethyl)glycine] and atrazine [2-chloro-

[1]Estimate from 1984 South Dakota Dept. of Ag. summary of acreage infestation by noxious weeds.

[2]Trade name Roundup, Monsanto Chemical Company, St. Louis, MO.

4-(ethylamino)-6-(isopropylamino)-s-triazine] plus crop oil. Because of the high dosage of atrazine required to control quackgrass, crop rotation is limited to corn or sorghum for several years after treatment due to residual soil activity. Glyphosate has no soil activity. Therefore, a producer is not limited in his crop selection following use of glyphosate. Control using recommended rates of glyphosate can cost up to $200 per hectare. This expense is prohibitive on marginally productive land.

Performance has been inconsistent with lower rates of glyphosate, and is diminished by high carrier volumes, hard water, low soil moisture, and combinations with some herbicides (2, 8, 15, 34, 41, 42). A means of increasing the effectiveness of glyphosate on quackgrass could provide significant economic benefit to producers.

The objectives of this research were to measure the economics of controlling leafy spurge in pasture using several common herbicide treatments, to characterize the interaction between ammonium sulfate and glyphosate for quackgrass control, and to determine the mechanism of ammonium sulfate interaction with glyphosate on increasing plant phytotoxicity.

LITERATURE REVIEW

Leafy spurge is a pernicious perennial that is competitive in both cropland and pastures due to rapid spread by seeds and rhizomes. Leafy spurge was first noted in the U.S. in 1827 and is present in 26 states (20). Seed is borne in capsules that dehisce when mature and can be displaced up to 5 m from the parent plant (5). Leafy spurge seed can remain dormant in the soil for up to 5 yr, and under adequate moisture will germinate throughout the growing season (55). Growth can also occur from adventitious buds present on roots. Shoots from root buds grew through 1 m of tamped soil within 12 months (55), which is indicative of the energy reserves stored in root tissue. Root buds germinated 3 m deep in pits dug for root studies (5), and shoots can grow from root segments as short as 1.25 cm (25). Plants achieve an average growth of 0.25 m height, a root depth of 1.1 m, and a 0.6 m diameter root spread at 3 months age (25). Leafy spurge can live 10 yr and develop roots 4.75 m deep (5).

Several studies have demonstrated that perennial weed control in pastures can increase forage production. Control of mixed stands of Canada thistle [*Cirsium arvense* (L.) Scop.] and musk thistle (*Carduus nutans* L.) in pasture resulted in 100 to 314% additional forage production during a 3 yr period of treatment (49). Perennial warm-season grass production increased 428 to 1440 kg/ha 7 months after herbicide applications in another study (43).

Cost of weed control and economic benefits from reducing weed competition with desirable vegetation are important considerations in selecting a weed control program. One difficulty with an economic analysis of weed control is assigning representative costs for herbicides used and production received. Prices vary considerably throughout a long-term study and can greatly influence profitability of treatments. Use of a sensitivity analysis in which returns are calculated for a range of herbicide or product prices can better determine herbicide treatments that are economically beneficial over a wide range of prices (36).

Quackgrass is an aggressive perennial grass that spreads by seeds and rhizomes. It is a native of Europe and was introduced into the northeast Unites States during the 1700's (47). Current distribution includes the northern half of the United States and southern Canada. One quackgrass plant was documented to produce 140 m of rhizomes with a root distribution 3.3 m in diameter its first year of growth. Dense stands of quackgrass can reduce corn yields up to 37% (65). Quackgrass seeds can successfully germinate through up to 10 cm of soil (47). Viable quackgrass seed was recovered after 10 yr of burial in the soil (60). New quackgrass plants can become established from rhizome segments less than 2 cm long (47). Cultivation may be used to control quackgrass by reducing stored food reserves in roots, drying rhizomes and topgrowth, and seasonally exposing rhizomes to freezing (13).

Use of cultivation as the sole means of controlling weed infestations in not always an effective alternative. During cultivation quackgrass infestations may be spread by unintentional transport of rhizome segments on tillage equipment. Also, cultivation must be performed every few weeks over one or more growing seasons to be to be effective as a perennial weed control measure. Such frequent tillage can result in increased soil erosion losses and high fuel and equipment costs. Herbicide applications for perennial weed control may assist or replace cultivation, with possible economic and conservation benefits.

Glyphosate is a non-specific herbicide which is absorbed by foliage of actively growing plants. The chemical name of glyphosate, N-(phosphonomethyl)glycine, indicates similarity to the amino acid glycine. Common uses in South Dakota are for control of volunteer corn and other tall weeds in soybeans, perennial weed control in fallow, and patch-killing weeds in pastures and shelterbelts. Since glyphosate is not selective in control, it must be used for fallow weed control or if used in actively growing crops applied only to the weeds. As a result, many applicators have been designed specifically for protecting a crop during glyphosate application. Because glyphosate translocates throughout an entire plant, perennial weeds can be effectively controlled with one application. Most other herbicides will damage only topgrowth, or topgrowth and part of the root system. Since most perennial plants regrow from root buds, many herbicides only delay their growth. Rate of kill with

glyphosate appears to be relatively slow. Often one week or more will elapse before injury symptoms are apparent, but in actuality the rate is much faster. Root death of quackgrass plants can be obtained even if topgrowth of treated plants is removed one day after glyphosate application (53).

Glyphosate is very slowly metabolized in treated plants. No metabolism was detected in purple nutsedge plants 16 days after application of glyphosate (66). Only an insignificant amount of glyphosate was metabolized in either common milkweed (Asclepias syriaca L.) or hemp dogbane (Apocynum cannabinum L.) plants grown in the greenhouse in another study (64). In a comparison of ^{14}C-labeled 2,4-D and glyphosate metabolism in hemp dogbane, 34 to 55% of applied ^{14}C-labeled 2,4-D was recovered as 2,4-D while 93 to 96% of applied ^{14}C-glyphosate was recovered as glyphosate in treated leaves 12 days after herbicide application (54).

Many factors can reduce weed control with glyphosate. Use of hard water in the spray solution has been demonstrated to decrease control of oat (Avena sativa L.) seedlings, barley (Hordeum vulgare L.), and volunteer wheat (Triticum aestivum L.) (8, 42, 48). Glyphosate treatments applied at high carrier volumes are less phytotoxic to oat seedlings and bermudagrass (Cynodon dactylon L.) than those applied at low carrier volumes (8, 32). This reduced control is believed to be due to reduced glyphosate concentration in spray droplets (1). Barnyardgrass (Echinochloa crus-galli L.) plants growing under moisture stress

are less susceptible to glyphosate than unstressed plants (2). In another study, absorption and translocation of ^{14}C-glyphosate was reduced in quackgrass plants growing at low temperatures or under moisture stress (34). When applied with either 2,4-D [(2,4-dichlorophenoxy)acetic acid] or bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), ^{14}C-glyphosate absorption and translocation was considerably reduced (41). This was believed to be due to less retention of glyphosate on treated leaves caused by solvents used in formulating 2,4-D or bromoxynil. Quackgrass control was less for plants treated at the two-leaf stage than with larger plants (53). Plant species vary in response to glyphosate treatment. Common milkweed (Asclepias syriaca L.) is more susceptible to glyphosate than is hemp dogbane (Apocynum cannabinum L.) (64).

While glyphosate has been studied since before 1970, the exact mechanisms of action have yet to be fully determined. Early research indicated one effect of glyphosate to be an inhibition of aromatic amino acid biosynthesis (31). The inhibition in duckweed (Lemna gibba L.) was somewhat offset by addition of L-phenylalanine to the culture medium. For Rhizobium japonicum, a nitrogen-fixing bacteria, the inhibition was best reversed by a combination of L-phenylalanine and L-tyrosine. An inhibition of chorismate mutase or prephenate dehydratase, two enzymes in the shikimic acid pathway of aromatic amino acid synthesis, was suggested. Later work by various researchers on microbes, whole plants, and plant tissue cultures produced

inconclusive results. Under some circumstances, glyphosate reduced phenylalanine synthesis while elevating tyrosine levels (21). Researchers hypothesized that growth symptoms observed following glyphosate treatment could be explained by an inhibition of indole-3-acetic acid production, which originates from tryptophan (6), or from toxic accumulations of ammonia (33). However, even though research results occasionally appear contradictory, they consistently point to an effect on the shikimic acid pathway of phenylalanine, tyrosine, and tryptophan synthesis.

Enzymes involved with the shikimic acid pathway are 5-dehydroquinate synthetase, shikimate dehydrogenase, shikimate kinase, 3-enolpyruvoylshikimate-5-phosphate synthetase, chorismate synthetase, tryptophan synthetase, chorismate mutase, prephenate dehydratase, and prephenate dehydrogenase (39). *In vitro* studies of glyphosate on 5-dehydroquinate synthetase, chorismate mutase, prephenate dehydratase, and prephenate dehydrogenase have indicated little or no direct effect on activities (29).

One recent theory to explain the reduction in aromatic amino acid content of glyphosate-treated plants is an increse in activity of phenylalanine ammonia-lyase (PAL). This enzyme along with tyrosine ammonia-lyase is involved in metabolism of phenylalanine and tyrosine. Hoagland and Duke published a series of seven articles from 1978 through 1981 on glyphosate effects on phenolic compounds. After initially finding an increase in PAL levels in maize roots following treatment with glyphosate, they

conducted a series of additional studies using soybean seedlings (16, 27). In a comparison of glyphosate, glycine, and glyphosine (a plant growth regulator structurally similar to glyphosate) effects on three-day old soybean seedlings (26), glyphosate caused the greatest increase in PAL activity over the three-day study period (17, 18, 28). Glyphosine increased PAL activity to a lesser extent, and glycine did not increase activity when compared to the control. Total soluble protein content was little affected by any treatment. Conclusions stated that glyphosate decreased soluble hydroxyphenolics, probably through depletion of the aromatic amino acid pool by increased PAL activity in synthesizing secondary phenolic compounds.

To determine if PAL enhancement is a common result of herbicide injury to soybean seedlings, an in vivo study of the effects of 16 herbicides representing 14 herbicide classes on growth and extractable PAL was conducted (29). Two herbicides other than glyphosate increased PAL activity, seven decreased PAL activity, and the rest had no effect.

Promotion of PAL activity by glyphosate application has also been substantiated by other researchers (11). A five-fold increase was detected in quackgrass rhizome nodes after 24 hr in glyphosate solution. However, addition of L-phenylalanine or PAL inhibitors did not alleviate glyphosate injury of wheat seedling root growth. Increased PAL activity in wheat root tips was also accompanied by increased chorismate mutase activity. The authors viewed the failure of PAL inhibitors to alleviate glyphosate

toxicity as an indication that PAL enhancement is an effect rather than a cause of phytotoxicity.

In one study, soybean seedling roots were fed glyphosate along with high levels of phenylalanine, tyrosine, and tryptophan to alleviate toxicity symptoms (19). A small (10%) but significant injury reduction was noted. The authors theorized that root-fed aromatic amino acids may be compartmentalized differently than endogenous amino acid pools affected by glyphosate. They stated that generally aromatic amino acids will substantially reverse the effects of glyphosate on growth of unicellular organisms or cell cultures of higher plants, while results with intact higher plants have been marginal.

Since the primary influence of glyphosate on the shikimic acid pathway is apparently not a depletion of aromatic amino acids through increased metabolism, the other probable explanation is an inhibition of amino acid synthesis as hypothesized by Jaworski (31). A group of West German researchers have recently found evidence to support this theory (4, 30). In studies of glyphosate effect on etiolated buckwheat seedlings, the accumulation of some phenylpropanoid substances was decreased 90% by glyphosate, while structurally similar compounds had little or no effect at equivalent concentrations. Of amino acids tested, only L-phenylalanine reduced the inhibition. Excised buckwheat hypocotyls accumulated shikimate to a 50-fold concentration increase over the control following 24-hr incubation in glyphosate solution. This was concurrent

with decreased concentration of the three aromatic amino acids, indicating an apparent block between shikimate and a common precursor of the amino acids, such as chorismate. In cultured cells of Galium mollugo, addition of chorismate offset glyphosate inhibition of anthraquinone production. Anthraquinone is a convenient indicator compound which is produced from chorismate by a different pathway than aromatic amino acids. The three enzymes involved in synthesis of chorismic acid from shikimic acid are shikimate kinase, 3-enolpyruvoylshikimate-5-phosphate, and chorismate synthetase. Studies showed shikimate kinase activity to be unaffected by glyphosate, so apparently one or both of the other enzymes is the target of glyphosate inhibition of the shikimic acid pathway.

It can be concluded that the primary target of glyphosate in the shikimic acid pathway lies between shikimate-5-phosphate and chorismic acid. This effectively blocks the production of phenylalanine, tyrosine, and tryptophan, which have chorismic acid as a common precursor. Concurrent with this inhibition, the PAL activity increases, resulting in further depletion of the aromatic amino acid pool. Injury symptoms in plants are probably partially due to lack of aromatic amino acids for biosynthetic processes, and possibly by increased ammonia levels from increased PAL activity. Studies are in progress to isolate the exact point of enzyme inhibition. The mechanism by which this inhibition occurs is thought to be through chelation

of divalent cobalt and magnesium, cofactors for enzymes involved in aromatic amino acid synthesis (19).

Early studies at South Dakota State University indicate that low rates of ammonium sulfate tank-mixed with glyphosate can improve quackgrass control over that from glyphosate alone. Enhancement of glyphosate phytotoxicity with ammonium sulfate has been documented for barley (42), quackgrass (7), and also Canada thistle [Cirsium arvense (L.) Scop.] (23). Ammonium sulfate with glyphosate at 0.2 kg/ha provided purple nutsedge (Cyperus rotundus L.) control equivalent to that from glyphosate alone at 0.8 kg/ha (59). Ammonium sulfate has also been documented to overcome deleterious effects of hard water on glyphosate activity (48).

Glyphosate is quite rapidly absorbed and translocated in plants. Sprankle et al. found that 34% of applied ^{14}C-glyphosate was absorbed by quackgrass in 4 hr, with 53% absorbed at 48 hr (56). However, cellular uptake of glyphosate is considerably less than would be expected based on chemical structure (52). Only 1.8% of applied ^{14}C-glyphosate was absorbed by detached hemp dogbane leaves over an 8 hr absorption period. Little absorption occurred after the first half-hour. In contrast, ^{14}C-leucine absorption increased linearly over a 2-hr absorption period. Since ^{14}C-leucine uptake indicated that cells were capable of absorbing for several hours, the authors hypothesized the cell membrane to act as the major barrier to glyphosate absorption in hemp dogbane.

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Many herbicides and other organic and inorganic substances have been demonstrated to increase plant cell membrane permeability (62). Paraquat (1,1'-dimethyl-4,4'-bipyridinium ion), diquat [6,7-dihydrodipyrido(1,2-a:2',1'-c)pyrazinediium ion], dinoseb (2-sec-butyl-4,6-dinitrophenol), and oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene] increased electrolyte leakage from bean (Phaseolus vulgaris L.) leaf discs after exposure for 12 hr or less (46). Leakage of electrolytes was considered an indicator of leaf cell membrane permeability. Glyphosate and several other herbicides did not alter bean leaf cell permeability in the same time period. Similar lack of glyphosate effect on membrane permeability was measured in red beet root tissue (Beta vulgaris L.) (22) and oats (62). Diquat and several surfactants were measured to increase membrane permeability in red beet root tissue (58). Another study measured increased cell membrane permeability in beans treated with linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methyl-urea], prometryn [2,4-bis(isopropylamino)-6-(methylthio)-s-triazine], sodium azide, and dalapon (2,2-dichloropropionic acid) 24 hr after treatment (12). Poovaiah and Leupold found ammonium sulfate to cause a considerable increase in betacyanin leakage from beet root slices while calcium chloride reduced leakage (44). Calcium chloride is a documented macromolecular destabilizer, while ammonium sulfate is an extreme stabilizer. The ammonium ion in a chloride solution produced a similar increase, but the sulfate ion as a potassium salt did not change

leakage when compared to distilled water. The authors suggested that these results reflect destabilization-stabilization effects of the solutes.

Conceivably, combinations of glyphosate with substances which increase cell membrane permeability could improve weed control with glyphosate, or enable use of glyphosate at lower rates. Improved control from use of ammonium sulfate with glyphosate could also reduce interference with glyphosate by antagonistic factors. Nothing is documented in the literature indicating whether ammonium sulfate in combination with glyphosate will influence plant cell membrane permeability. Also, no researcher has published any theories on the mechanism of ammonium sulfate enhancement of glyphosate. Therefore, based on previous tests which demonstrate an increase in membrane permeability from ammonium sulfate, one possible mechanism of glyphosate enhancement by ammonium sulfate is through increased glyphosate absorption due to ammonium sulfate-induced changes in cell membrane permeability.

The objectives for this research were to:

1) evaluate the efficacy and economics of several herbicide treatments for leafy spurge control in pasture.
2) characterize the interaction between ammonium sulfate and glyphosate for quackgrass control, and determine whether very low rates of selected herbicides will enhance glyphosate for quackgrass control.

3) determine the physiological effects of ammonium sulfate and glyphosate combinations on quackgrass by measuring absorption, translocation, and ultrastructure changes.

MATERIALS AND METHODS

Efficacy and economics of leafy spurge control

General. A field experiment was established on a Clarno- (Typic Haplustoll; fine-loamy, mixed, mesic) Bonilla (Pachic Haplustoll; fine-loamy, mixed, mesic) loam in a pasture near Woonsocket, South Dakota in 1978. Leafy spurge occurred uniformly throughout the pasture with a density of approximately 100 plants/m^2 at study initiation. Kentucky bluegrass (*Poa pratensis* L.) was the primary forage component of the pasture. Smooth bromegrass (*Bromus inermis* Leyss.), switchgrass (*Panicum virgatum* L.), slender wheatgrass (*Agropyron trachycaulum* L.), big bluestem (*Andropogon gerardi* Vitman), and little bluestem (*Andropogon scoparius* Michx.), were also present. The study area was fenced to prevent grazing.

The experiment was designed as a randomized complete block consisting of four replications of 18 treatments in plots 6.1 by 12.2 m. Treatments consisted of spring or spring and fall applications of the butoxyethanol ester of 2,4-D [(2,4-dichlorophenoxy)acetic acid] alone or in combination with the dimethylamine salt of dicamba (3,6-dichloro-o-anisic acid), the potassium salt of picloram (4-amino-3,5,6-trichloropicolinic acid), or the sodium salt of glyphosate [N-(phosphonomethyl)-glycine]. All herbicide rates are expressed as kg ae/ha. Treatments were applied to the study on May 31, 1978, September 23, 1978, June 12, 1979, October 2, 1979, June 28, 1980, September 12, 1980,

June 23, 1981, September 17, 1981, June 3, 1982, September 21, 1982, and June 9, 1983. Leafy spurge was blooming when treatments were applied in the spring and postblooming when treatments were applied in the fall. Herbicides were applied in 187 L/ha water at 276 kPa pressure with flat-fan spray nozzles using a tractor-mounted sprayer. Analysis of variance was performed on all data, and means were separated using the Waller-Duncan k-ratio t-test (57) at k = 100 (P = 0.05).

Leafy spurge control. Leafy spurge control was visually evaluated in 1979 through 1983 when spring treatments were applied using a 0 to 100% scale in which 0 represented no control and 100 represented complete control of topgrowth. Orthogonal contrasts were made for each year comparing spring applications of 2,4-D at 1.7 and 3.4 kg/ha vs. spring and fall applications of 2,4-D at 0.8 and 1.7 kg/ha.

Forage and leafy spurge production. The area was mowed late fall of 1981 and 1982 to remove existing topgrowth prior to yield measurements the following year. Total dry production was determined by harvesting and weighing a fresh sample from a 0.6- by 12.2-m area in each plot on August 10, 1982 and August 17, 1983. A 150-g subsample was removed, oven dried, and reweighed to measure moisture content. An area 1 m^2 was also harvested from each plot at the same time for later drying and separation into forage and leafy spurge fractions. Dry-forage and leafy spurge yields were calculated for all plots based on total

production and relative forage and leafy spurge content in each sample.

Economics of leafy spurge control. Economic comparisons between treatments are presented as net marginal returns. Forage value of untreated plots is the baseline from which all marginal returns are calculated. Therefore, the marginal return is the value of additional forage obtained due to treatment. Marginal costs are the additional costs incurred due to treatment. Thus net marginal return was calculated for each plot for 1982 and 1983 using the formula: marginal return = forage increase over untreated x forage value - mean annual treatment cost (63). Forage value was calculated using the 1982 and 1983 average monthly price of $45.00 per 1000 kg. Herbicide costs represent an average of 1979 and 1982 retail prices, and were: 2,4-D $5.70/kg ae, dicamba $22.55/kg ae, picloram $93.17/kg ae, and glyphosate $49.93/kg ae. Cost to make one application was assigned $6.00/ha. Marginal cost for the 1982 and 1983 harvest was calculated by dividing total treatment cost by the number of years since study initiation. For example, 2,4-D applied at 0.84 kg/ha spring and fall had a mean annual treatment cost of $21.58 at the 1982 harvest. This was calculated by dividing the total cost of herbicide and application for nine applications (spring 1978 to spring 1982) of $97.07 by 4.5 years. Thus, marginal costs were assigned on the assumption that forage yields each year were influenced by the cumulative result of treatments since the beginning of the study. This method over-estimates average